# Metaphase preparation from murine bone marrow

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**Method Article**

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# Abstract

# Introduction

Genomic instabilities including chromosomal translocations are frequently associated with genetic diseases and cancer, especially leukemia. Cytogenetic studies of these diseases requiring preparation of metaphase chromosomes are often key to revealing their chromosomal abnormalities. Treatment of in vitro disease cell cultures with cell cycle inhibitors, e.g. colcemid, have proved to be a very simple and effective metaphase preparation method for cytogenetic studies. Two good examples are metaphase preparation from cultured cells and some types of myeloid leukemia. However, this approach has some limitions and disadvantages: 1) it is difficult to grow many types of primary cell in vitro; 2) in vitro cultures tend to introduce additional artificial mutations to the genome. To overcome these issues, we have established an approach to prepare metaphase chromosomes directly from murine bone marrow. With this new approach, we were able to get 5-10% of metaphase chromosomes from primary bone marrow leukocytes and identify chromosomal translocations recurring in a Pten null murine model developing T cell leukemia \(Figure 1,2 \[1]).

# Reagents

1. 200 µg/mL colcemid \(demecolcine, Sigma 27645, dissolved in 1x PBS, aliquotes are stored at -20°C) 2. 0.06 M potassium chloride \(hypotonic solution) 3. Fixative solution \(methanol \[100%]:acetic acid \[glacial]=3:1, fresh preparation required) 4. 20x Giemsa stain solution \(Sigma GS500, freshly diluted) 5. 40 µm cell strainer \(BD) 6. Pre-cleaned slides

# Equipment

1. 37°C water bath 2. 37°C incubator 3. slow rotator 3. gas or alcohol burner 4. phase-contrast microscope

# Procedure

1. Warm 0.06M potassium chloride in a 37°C water bath. 2. Inject 250µL of 200µg/mL colcemid into the peritoneum of mice and arrest cell cycle for 30 minutes. 3. Flush bone marrow cells with 30mL pre-warmed 0.06M potassium chloride into 50mL tube through 40µm cell strainer and rock sample tubes gently in 37°C incubator for 20 min. 4. Make fixative solution \(methanol \[100%]:glacial acetic acid=3:1) during hypotonic incubation. 5. Add 2mL Fixative into samples, mix gently. Centrifuge at 1500rpm for 7 min and discard supernatant. 6. Resuspend cells thoroughly with 0.5mL 0.06M potassium chloride. 7. Add few drops of fixative to cells and mix well. Keep adding and mixing fixative drops into cells until reaching 3mL. Add fixative up to 20mL in total, mix by inversion, and incubate at room temperature for 10 min. 8. Run the sample mixtures through 40µm cell strainer. Centrifuge at 1500rpm for 7 min and discard supernatant. 9. Resuspend pellets in 20 mL fixative and incubate at room temperature for 10 min.

Centrifuge at 1500rpm for 7 min and discard supernatant. 10. Repeat step 9 once. 11. Resuspend cell pellets in 2mL fixative and store at -20°C for long-term storage. 12. To determine chromosome quality, use the flame method or other ways to spread and stain chromosomes on slides. Drop 30μL fixed cells onto pre-cleaned and wet slides tilted at a 45 degree angle. Put slides in burner flame for 2 seconds to allow chromosomes to spread out. Let slides dry. 13. dilute 20x Giemsa stain with water to 1x Giemsa solution. Stain chromosome spread slides with 1x Giemsa solution for 5 minutes. Wash off Giemsa solution and let slides dry. Check chromosome quality under microscope.

## Timing

It takes 2 hour to perform the experiment.

## Critical Steps

Step 7. The first few rounds of adding and mixing fixative drops are very critical for chromosome quality. Step 8. It is very important to remove cell debris through 40μm cell strainer beside filtering at Step 3. Step 12. Slides have to be rinsed and cleaned in distilled water before use. Otherwise, the quality of the Giemsa-stained slides will be compromised. Step 13. Stain chromosome slides with 1x Giesma solution for 5 minutes at most. Excess staining time tends to result in overdarkened chromosomes.

## Troubleshooting

1. Few chromosome spreads are available on slides. This is due to poor chromosome spreading by the flame method \(Step 12). More practice runs should be conducted to improve spreading techniques. Alternative methods, such as dropping onto ice-cold wet slides from 30cm higher, are available for use, too.

## Anticipated Results

1. A good chromosome slide by flame method should show 30-60% of chromosome spreads. 2. A good preparation should show that metaphases constitute approximately 5-10% of bone marrow chromosome spreads.

## References

1. Guo, W, _et al_. \(2008). Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature **453**, 529-533.

## Acknowledgements

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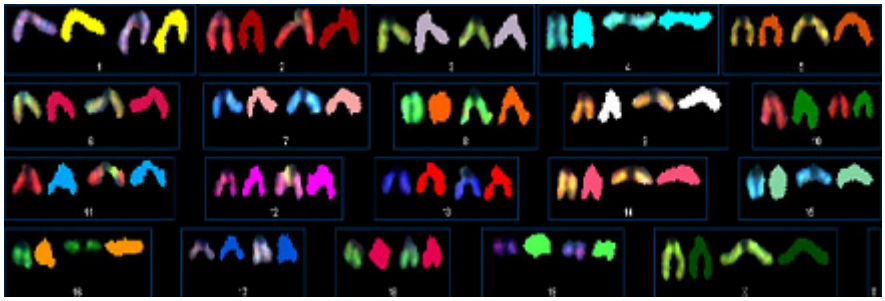
# Figures



### Figure 1

Spectral karyotyping (SKY) analysis of wild-type metaphases Metaphases were prepared directly from bone marrow of a wild type mouse. No abnormality was detected. A larger version of this figure can be found "here":http://protocols.nature.com/image/show/990

### Figure 2

SKY analysis of T cell leukemia metaphases Metaphases were prepared directly from bone marrow of a Pten null T cell leukemia mouse. T(14;15) was detected and hightlighted in white box. A larger version of this figure can be found "here":http://protocols.nature.com/image/show/996